

What is claimed is:

1. A molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like domain 1/domain 2 interface, wherein the domain 1/domain 2 interface comprises amino acids 205-209, 232-238, 415-420 and 460-467, the domain 1/domain 2 interface being defined by a set of points having a root mean square deviation of less than about 1.5Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed in Tables 1, 2, or 3 respectively.

2. A molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like oligonucleotide binding site, wherein the oligonucleotide binding site comprises amino acids selected from the group consisting of (1) domain 1 oligonucleotide binding site amino acids 230-232, 255, 269, and 270-272, and (2) domain 2 oligonucleotide binding site amino acids 391-393, 411-413, 415, 416 and 460; the oligonucleotide binding site being defined by a set of points having a root mean square deviation of less than about 1.5Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed in Tables 1, 2, or 3 respectively.

3. A Hepatitis C virus helicase molecule or molecular complex comprising at least a first and a second oligonucleotide binding site, wherein the distance between the first and the second oligonucleotide binding sites is less than about 21 angstroms.

4. The Hepatitis C virus helicase molecule or molecular complex of claim 3, wherein the distance between the first and the second oligonucleotide binding sites is about 18.8 to about 19.5 angstroms.

5. A molecule or molecular complex that is structurally homologous to a Hepatitis C virus helicase molecule or molecular complex, wherein the Hepatitis C virus helicase molecule or

molecular complex is represented by at least a portion of the structure coordinates listed in Tables 1, 2, or 3.

6. A scalable three-dimensional configuration of points, at least a portion of said points derived from structure coordinates of at least a portion of a Hepatitis C virus helicase molecule or molecular complex as listed in Tables 1, 2, or 3 and comprising at least one of a Hepatitis C virus helicase or Hepatitis C virus helicase-like domain 1/domain 2 interface, domain 1 oligonucleotide binding site, or domain 2 oligonucleotide binding site.

7. The scalable three-dimensional configuration of points of claim 6, wherein substantially all of said points are derived from structure coordinates of a Hepatitis C virus helicase molecule or molecular complex as listed in Tables 1, 2, or 3.

8. The scalable three-dimensional configuration of points of claim 6 wherein at least a portion of the points derived from the Hepatitis C virus helicase structure coordinates are derived from structure coordinates representing the locations of at least the backbone atoms of amino acids selected from the group consisting of (1) domain 1/domain 2 interface amino acids 205-209, 232-238, 415-420 and 460-467, (2) domain 1 oligonucleotide binding site amino acids 230-232, 255, 269, and 270-272, and (3) domain 2 oligonucleotide binding site amino acids 391-393, 411-413, 415, 416 and 460, as represented by structure coordinates of UHCV-A, UHCV-B, or UHHO in Tables 1, 2, and 3 respectively.

9. The scalable three-dimensional configuration of points of claim 6 displayed as a holographic image, a stereodiagram, a model or a computer-displayed image.

10. A scalable three-dimensional configuration of points, at least a portion of the points derived from structure coordinates of at least a portion of a molecule or a molecular complex that is structurally homologous to a Hepatitis C virus helicase molecule or molecular complex and comprises at least one of a Hepatitis C virus helicase or Hepatitis C virus helicase-like domain 1/domain 2 interface, domain 1 oligonucleotide binding site, or domain 2 oligonucleotide binding site.

11. The scalable three-dimensional configuration of points of claim 10 displayed as a holographic image, a stereodiagram, a model or a computer-displayed image.

5 12. A machine-readable data storage medium comprising a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using said data, is capable of displaying a graphical three-dimensional representation of at least one molecule or molecular complex selected from the group consisting of:

10 (i) a molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like domain 1/domain 2 interface, wherein the domain 1/domain 2 interface comprises amino acids 205-209, 232-238, 415-420 and 460-467, the domain 1/domain 2 interface being defined by a set of points having a root mean square deviation of less than about 1.5 Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed
15 in Tables 1, 2, or 3 respectively;

(ii) a molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like oligonucleotide binding site, wherein the oligonucleotide binding site comprises amino acids selected from the group consisting of (1) domain 1 oligonucleotide binding site amino acids 230-232, 255, 269, and 270-272, and (2)
20 domain 2 oligonucleotide binding site amino acids 391-393, 411-413, 415, 416 and 460; the oligonucleotide binding site being defined by a set of points having a root mean square deviation of less than about 1.5 Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed in Tables 1, 2, or 3 respectively;

25 (iii) a Hepatitis C virus helicase molecule or molecular complex comprising at least a first and a second oligonucleotide binding site, wherein the distance between the first and the second oligonucleotide binding sites is less than about 21 angstroms; and

(iv) a molecule or molecular complex that is structurally homologous to a Hepatitis C virus helicase molecule or molecular complex, wherein the Hepatitis C virus helicase
30 molecule or molecular complex is represented by at least a portion of the structure coordinates listed in Tables 1, 2, or 3.

13. A machine-readable data storage medium comprising a data storage material encoded with a first set of machine readable data which, when combined with a second set of machine readable data, using a machine programmed with instructions for using said first set of data and said second set of data, can determine at least a portion of the structure coordinates corresponding to the second set of machine readable data, wherein said first set of data comprises a Fourier transform of at least a portion of the structure coordinates for Hepatitis C virus helicase listed in Tables 1, 2, or 3; and said second set of data comprises an x-ray diffraction pattern of a molecule or molecular complex of unknown structure.

14. A method for obtaining structural information about a molecule or a molecular complex of unknown structure comprising:

crystallizing the molecule or molecular complex;

generating an x-ray diffraction pattern from the crystallized molecule or molecular complex;

applying at least a portion of the structure coordinates set forth in Tables 1, 2, or 3 to the x-ray diffraction pattern to generate a three-dimensional electron density map of at least a portion of the molecule or molecular complex whose structure is unknown.

15. A method for homology modeling a Hepatitis C virus helicase homolog comprising:

aligning the amino acid sequence of a Hepatitis C virus helicase homolog with an amino acid sequence of Hepatitis C virus helicase (SEQ ID NO: 1) and incorporating the sequence of the Hepatitis C virus helicase homolog into a model of Hepatitis C virus helicase derived from structure coordinates set forth in Tables 1, 2, or 3 to yield a preliminary model of the

Hepatitis C virus helicase homolog;

subjecting the preliminary model to energy minimization to yield an energy minimized model;

remodeling regions of the energy minimized model where stereochemistry restraints are violated to yield a final model of the Hepatitis C virus helicase homolog.

16. A computer-assisted method for identifying an inhibitor of Hepatitis C virus helicase activity comprising:

supplying a computer modeling application with a set of structure coordinates for at least a portion of at least one molecule or molecular complex selected from the group consisting of:

(i) a molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like domain 1/domain 2 interface, wherein the domain 1/domain 2 interface comprises amino acids 205-209, 232-238, 415-420 and 460-467, the domain 1/domain 2 interface being defined by a set of points having a root mean square deviation of less than about 1.5Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed in Tables 1, 2, or 3 respectively;

(ii) a molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like oligonucleotide binding site, wherein the oligonucleotide binding site comprises amino acids selected from the group consisting of (1) domain 1 oligonucleotide binding site amino acids 230-232, 255, 269, and 270-272, and (2) domain 2 oligonucleotide binding site amino acids 391-393, 411-413, 415, 416 and 460; the oligonucleotide binding site being defined by a set of points having a root mean square deviation of less than about 1.5Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed in Tables 1, 2, or 3 respectively;

(iii) a Hepatitis C virus helicase molecule or molecular complex comprising at least a first and a second oligonucleotide binding site, wherein the distance between the first and the second oligonucleotide binding sites is less than about 21 angstroms; and

(iv) a molecule or molecular complex that is structurally homologous to a Hepatitis C virus helicase molecule or molecular complex, wherein the Hepatitis C virus helicase molecule or molecular complex is represented by at least a portion of the structure coordinates listed in Tables 1, 2, or 3;

wherein said portion of the molecule comprises at least one HCV binding site selected from the group consisting of an oligonucleotide binding site on domain 1, an oligonucleotide binding site on domain 2, an NTP binding site, and a domain 1/domain 2 interface;

supplying the computer modeling application with a set of structure coordinates of a chemical entity; and

determining whether the chemical entity is expected to bind to or interfere with the molecule or molecular complex.

5

17. The method of claim 16 wherein determining whether the chemical entity is expected to bind to or interfere with the molecule or molecular complex comprises performing a fitting operation between the chemical entity and a binding site of the molecule or molecular complex, followed by computationally analyzing the results of the fitting operation to quantify the association between the chemical entity and the binding site.

10

18. The method of claim 16 further comprising screening a library of chemical entities.

15

19. A computer-assisted method for designing an inhibitor of Hepatitis C virus helicase activity comprising:

supplying a computer modeling application with a set of structure coordinates of at least a portion of at least one molecule or molecular complex selected from the group consisting of:

20

(i) a molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like domain 1/domain 2 interface, wherein the domain 1/domain 2 interface comprises amino acids 205-209, 232-238, 415-420 and 460-467, the domain 1/domain 2 interface being defined by a set of points having a root mean square deviation of less than about 1.5Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed in Tables 1, 2, or 3 respectively;

25

(ii) a molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like oligonucleotide binding site, wherein the oligonucleotide binding site comprises amino acids selected from the group consisting of (1) domain 1 oligonucleotide binding site amino acids 230-232, 255, 269, and 270-272, and (2) domain 2 oligonucleotide binding site amino acids 391-393, 411-413, 415, 416 and 460; the oligonucleotide binding site being defined by a set of points having a root mean square

30

deviation of less than about 1.5Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed in Tables 1, 2, or 3 respectively;

(iii) a Hepatitis C virus helicase molecule or molecular complex comprising at least a first and a second oligonucleotide binding site, wherein the distance between the first and the second oligonucleotide binding sites is less than about 21 angstroms; and

(iv) a molecule or molecular complex that is structurally homologous to a Hepatitis C virus helicase molecule or molecular complex, wherein the Hepatitis C virus helicase molecule or molecular complex is represented by at least a portion of the structure coordinates listed in Tables 1, 2, or 3;

wherein said portion of the molecule comprises at least one HCV binding site selected from the group consisting of an oligonucleotide binding site on domain 1, an oligonucleotide binding site on domain 2, an NTP binding site, and a domain 1/domain 2 interface;

supplying the computer modeling application with a set of structure coordinates for a chemical entity;

evaluating the potential binding interactions between the chemical entity and substrate binding site of the molecule or molecular complex;

structurally modifying the chemical entity to yield a set of structure coordinates for a modified chemical entity; and

determining whether the modified chemical entity is an inhibitor expected to bind to or interfere with the molecule or molecular complex, wherein binding to or interfering with the molecule or molecular complex is indicative of potential inhibition of Hepatitis C virus helicase activity.

20. The method of claim 19 wherein determining whether the modified chemical entity is an inhibitor expected to bind to or interfere with the molecule or molecular complex comprises performing a fitting operation between the chemical entity and a binding site of the molecule or molecular complex, followed by computationally analyzing the results of the fitting operation to quantify the association between the chemical entity and the binding site.

21. The method of claim 19 wherein the set of structure coordinates for the chemical entity is obtained from a chemical fragment library.

22. A computer-assisted method for designing an inhibitor of Hepatitis C virus helicase activity de novo comprising:

supplying a computer modeling application with a set of structure coordinates of at least a portion of at least one molecule or molecular complex selected from the group consisting of:

(i) a molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like domain 1/domain 2 interface, wherein the domain 1/domain 2 interface comprises amino acids 205-209, 232-238, 415-420 and 460-467, the domain 1/domain 2 interface being defined by a set of points having a root mean square deviation of less than about 1.5Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed in Tables 1, 2, or 3 respectively;

(ii) a molecule or molecular complex comprising at least a portion of a Hepatitis C virus helicase or Hepatitis C virus helicase-like oligonucleotide binding site, wherein the oligonucleotide binding site comprises amino acids selected from the group consisting of (1) domain 1 oligonucleotide binding site amino acids 230-232, 255, 269, and 270-272, and (2) domain 2 oligonucleotide binding site amino acids 391-393, 411-413, 415, 416 and 460; the oligonucleotide binding site being defined by a set of points having a root mean square deviation of less than about 1.5Å from points representing the backbone atoms of said amino acids as represented by the structure coordinates of UHCV-A, UHCV-B, or UHHO as listed in Tables 1, 2, or 3 respectively;

(iii) a Hepatitis C virus helicase molecule or molecular complex comprising at least a first and a second oligonucleotide binding site, wherein the distance between the first and the second oligonucleotide binding sites is less than about 21 angstroms; and

(iv) a molecule or molecular complex that is structurally homologous to a Hepatitis C virus helicase molecule or molecular complex, wherein the Hepatitis C virus helicase molecule or molecular complex is represented by at least a portion of the structure coordinates listed in Tables 1, 2, or 3;

wherein said portion of the molecule comprises at least one HCV binding site selected from the group consisting of an oligonucleotide binding site on domain 1, an oligonucleotide binding site on domain 2, an NTP binding site, and a domain 1/domain 2 interface;

5 computationally building a chemical entity represented by set of structure coordinates;
and

determining whether the chemical entity is an inhibitor expected to bind to or interfere with the molecule or molecular complex, wherein binding to or interfering with the molecule or molecular complex is indicative of potential inhibition of Hepatitis C virus helicase activity.

10

23. The method of claim 22 wherein determining whether the chemical entity is an inhibitor expected to bind to or interfere with the molecule or molecular complex comprises performing a fitting operation between the chemical entity and a binding site of the molecule or molecular complex, followed by computationally analyzing the results of the fitting operation to quantify the association between the chemical entity and the binding site.

15

24. The method of any of claims 16, 19, or 22 further comprising supplying or synthesizing the potential inhibitor, then assaying the potential inhibitor to determine whether it inhibits Hepatitis C virus helicase activity.

20

25. A method for making an inhibitor of Hepatitis C virus helicase activity, the method comprising chemically or enzymatically synthesizing a chemical entity to yield an inhibitor of Hepatitis C virus helicase activity, the chemical entity having been identified during a computer-assisted process comprising supplying a computer modeling application with a set of structure coordinates of a molecule or molecular complex, the molecule or molecular complex comprising at least a portion of at least one of a Hepatitis C virus helicase or Hepatitis C virus helicase-like binding site; supplying the computer modeling application with a set of structure coordinates of a chemical entity; and determining whether the chemical entity is expected to bind to or interfere with the molecule or molecular complex at a binding site, wherein binding to or interfering with the molecule or molecular complex is indicative of potential inhibition of Hepatitis C virus helicase activity.

25

30

26. A method for making an inhibitor of Hepatitis C virus helicase activity, the method comprising chemically or enzymatically synthesizing a chemical entity to yield an inhibitor of Hepatitis C virus helicase activity, the chemical entity having been designed during a computer-assisted process comprising supplying a computer modeling application with a set of structure coordinates of a molecule or molecular complex, the molecule or molecular complex comprising at least a portion of at least one of a Hepatitis C virus helicase or Hepatitis C virus helicase-like binding site; supplying the computer modeling application with a set of structure coordinates for a chemical entity; evaluating the potential binding interactions between the chemical entity and a binding site of the molecule or molecular complex; structurally modifying the chemical entity to yield a set of structure coordinates for a modified chemical entity; and determining whether the chemical entity is expected to bind to or interfere with the molecule or molecular complex at the binding site, wherein binding to or interfering with the molecule or molecular complex is indicative of potential inhibition of Hepatitis C virus helicase activity.

27. A method for making an inhibitor of Hepatitis C virus helicase activity, the method comprising chemically or enzymatically synthesizing a chemical entity to yield an inhibitor of Hepatitis C virus helicase activity, the chemical entity having been designed during a computer-assisted process comprising supplying a computer modeling application with a set of structure coordinates of a molecule or molecular complex, the molecule or molecular complex comprising at least a portion of at least one of a Hepatitis C virus helicase or Hepatitis C virus helicase-like binding site; computationally building a chemical entity represented by set of structure coordinates; and determining whether the chemical entity is expected to bind to or interfere with the molecule or molecular complex at a binding site, wherein binding to or interfering with the molecule or molecular complex is indicative of potential inhibition of Hepatitis C virus helicase activity.

28. An inhibitor of Hepatitis C virus helicase activity identified, designed or made according to the method of any of the claims 16, 19, 22, 25, 26, and 27.

29. A composition comprising an inhibitor of Hepatitis C virus helicase activity identified or designed according to the method of any of the claims 16, 19, 22, 25, 26, and 27.

30. A pharmaceutical composition comprising an inhibitor of Hepatitis C virus helicase activity identified or designed according to the method of any of the 16, 19, 22, 25, 26, and 27 or a salt thereof, and pharmaceutically acceptable carrier.

31. A method for crystallizing a Hepatitis C virus helicase molecule or molecular complex comprising growing a crystal from a precipitant solution comprising purified Hepatitis C virus helicase, about 3% by weight to about 14% by weight PEG, about 5% by weight to about 15% by weight DMSO, and about 0.05M to about 0.07M potassium phosphate.

32. A method for co-crystallizing a Hepatitis C virus helicase molecule and a ligand to yield a molecular complex, comprising:

exchanging purified Hepatitis C virus helicase into a solution comprising HEPES, EDTA, and dithiothreitol;

concentrating the Hepatitis C virus helicase to a concentration of about 12-16mg/mL;

combining concentrated Hepatitis C virus helicase with the ligand in a mixture comprising about 4% by weight to about 14% by weight PEG and about 5% by weight to about 15% by weight DMSO; and

growing a co-crystal by vapor diffusion.

33. The method of claim 32 wherein combining the concentrated Hepatitis C virus helicase with the ligand in a mixture comprising PEG and DMSO and growing the co-crystal are performed in the absence of potassium phosphate.

34. The method of claim 32 wherein the ligand binds to an NTP binding site on the Hepatitis C virus helicase.

35. A method for crystallizing a Hepatitis C virus helicase molecule or molecular complex comprising growing a crystal by vapor diffusion with macro-seeding from a precipitant

solution comprising purified Hepatitis C virus helicase, HEPES, and about 4% by weight to about 14% by weight mono-alkyl ether of PEG.

36. A method for co-crystallizing a Hepatitis C virus helicase molecule and a ligand to yield a molecular complex, comprising growing a crystal by vapor diffusion with macro-seeding from a precipitant solution comprising purified HCV helicase, HEPES, about 4% by weight to about 14% by weight mono-alkyl ether of PEG, and the ligand, wherein the ligand binds to at least one oligonucleotide binding site on the Hepatitis C virus helicase.

37. The method of claims 31-36 wherein the amino acid sequence of the Hepatitis C virus helicase is SEQ ID NO:1.

38. Crystalline Hepatitis C virus helicase comprising a tetragonal crystal having unit cell dimensions of $a = b = 109 \text{ \AA} \pm 3 \text{ \AA}$; $c = 84 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and space group $P4_1$; the unit cell containing two molecules in an asymmetric unit.

39. The crystalline Hepatitis C virus helicase of claim 38 wherein the amino acid sequence of Hepatitis C virus helicase is SEQ ID NO:1.

40. Crystalline Hepatitis C virus helicase comprising an orthorhombic crystal characterized by unit cell dimensions of $a = 66 \text{ \AA} \pm 2 \text{ \AA}$; $b = 110 \text{ \AA} \pm 3 \text{ \AA}$; $c = 64 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and a space group $P2_12_12_1$; the unit cell containing one molecule in the asymmetric unit.

41. The crystalline Hepatitis C virus helicase of claim 40 wherein the amino acid sequence of Hepatitis C virus helicase is SEQ ID NO:1.

42. Crystalline Hepatitis C virus helicase having an amino acid sequence is SEQ ID NO:1.

43. A composition comprising crystalline Hepatitis C virus helicase of any of claims 38-42.

44. A method for solving a crystal structure of a crystal of Hepatitis C virus helicase having unit cell dimensions of $a = b = 109 \text{ \AA} \pm 3 \text{ \AA}$; $c = 84 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and space group $P4_1$, the unit cell containing two molecules in an asymmetric unit, the method comprising:

- 5 generating an x-ray diffraction pattern from the crystal,
collecting diffraction data, and
analyzing the data to generate the structure coordinates for the Hepatitis C virus helicase.

10 45. A method for solving a crystal structure of a crystal of Hepatitis C virus helicase having unit cell dimensions of $a = 66 \text{ \AA} \pm 2 \text{ \AA}$; $b = 110 \text{ \AA} \pm 3 \text{ \AA}$; $c = 64 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and a space group $P2_12_12_1$, the unit cell containing one molecule in an asymmetric unit, the method comprising:

- 15 generating an x-ray diffraction pattern from the crystal,
collecting diffraction data, and
analyzing the data to generate the structure coordinates for the Hepatitis C virus helicase.

20 46. The method of claims 44 or 45 wherein the amino acid sequence of the Hepatitis C virus helicase is SEQ ID NO:1.

47. A method for incorporating a chemical entity in a crystal comprising placing a tetragonal crystal of Hepatitis C virus helicase having unit cell dimensions of $a = b = 109 \text{ \AA} \pm 3 \text{ \AA}$; $c = 84 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and space group $P4_1$ in an aqueous solution comprising
25 about 1mM to about 10mM chemical entity, and 0% by weight to about 15% by weight DMSO.

48. A method for incorporating a chemical entity in a crystal comprising placing an orthorhombic crystal of Hepatitis C virus helicase having unit cell dimensions of $a = 66 \text{ \AA} \pm 2 \text{ \AA}$; $b = 110 \text{ \AA} \pm 3 \text{ \AA}$; $c = 64 \text{ \AA} \pm 2 \text{ \AA}$; $\alpha = \beta = \gamma = 90^\circ$; and a space group $P2_12_12_1$ in an
30

add
 a^7

65